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# Effect of feeding and male presence on some biological characteristics of female *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae)

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**Abstract**

Low prominence of natural enemies can cause suboptimal success in biological control programs. In present study, the effects of honey feeding and the availability of males were studied on the biological characteristics of *Trichogramma brassicae* Bezdenko. Four treatments were considered: male was available for female for merely 24 h, either with or without honey; male was continuously presented for females, either with or without honey. Twenty pairs (1 female × 1 male) of newly emerged parasitoids were placed in glass tubes and considered as the treatments. Mortality of females was recorded daily in order to indicate longevity. To determine fecundity, the emergence rate and female progeny percentage, 150 fresh *Sitotroga cerealella* Olivier eggs were provided for each female in the entire treatments on a daily basis. Results indicated that interaction between adult nutrition and male presence was insignificant ($P > 0.01$) and did not influence any of biological characteristics. Adult nutrition was more important than male presence. Honey-fed females lived 5-folds longer than the unfed females. Longevity was not significantly influenced by male presence. Fecundity of honey-fed females was 67 and 70.2 eggs; however, 18.4 and 19 eggs for unfed females. Continuous presence of male did not change fecundity. There were no significant differences in the emergence rate in all treatments and it was more than 90% for the entire treatments. Long-lived females produced more males. Percentage of female progeny was not influenced by period of male presence. Therefore, quality of parasitoids was more persuaded by diet provided rather than the period of male presence.

**Key words:** *Trichogramma brassicae*, nutrition, male presence, longevity, fecundity, emergence rate

**چکیده**

کیفیت پایین دشمنان طبیعی در شکست برنامه‌های کنترل بیولوژیک نقش دارد. در پژوهش حاضر تأثیر تغذیه از عسل و مدت زمان حضور نرها بر برخی خصوصیات زیستی زنبور پارازیتوئید *Trichogramma brassicae* Bezdenko مورد مطالعه قرار گرفت. چهار تیمار عبارت بودند از: حضور پارازیتوئید نر به مدت ۲٤ ساعت همراه با پارازیتوئید ماده با عسل و بدون عسل؛ حضور پارازیتوئید نر برای تمام عمر همراه با پارازیتوئید ماده با عسل و بدون عسل. در هر تیمار بیست جفت زنبور پارازیتوئید تازه‌خارج‌شده به‌صورت جداگانه به لوله‌ی آزمایش منتقل شدند. میزان مرگ و میر پارازیتوئید ماده به طور روزانه ثبت گردید. به منظور تعیین میزان باروری، نرخ خروج و درصد نتاج ماده، ۱۵۰ عدد تخم تازه‌ی شب‌پره‌ی *Sitotroga cerealella* Olivier برای هر تیمار به‌صورت روزانه تأمین شد. نتایج نشان داد که اثر متقابل بین عامل تغذیه و مدت زمان حضور نرها معنی‌دار نبوده ($P<0/01$) و تأثیری بر هیچ‌یک از خصوصیات زیستی تریکوگراما ندارد. تغذیه نقش بسیار مهم‌تری نسبت به مدت زمان حضور نرها داشت. طول عمر پارازیتوئیدهای ماده در تیمارهای حاوی عسل ۵ برابر تیمارهای بدون تغذیه بود. مدت زمان حضور نرها بر طول عمر تأثیر معنی‌داری نداشت. میزان تولید تخم در تیمارهای حاوی عسل ٦۷ و ۷۰/۲ عدد در مقایسه با ۱۸/٤ و ۱۹ عدد در تیمارهای بدون عسل بود. میزان تولید تخم تحت تأثیر مدت زمان حضور نرها نبود. در کلیه‌ی تیمارها، نرخ خروج اختلاف معنی‌داری نداشت و بیشتر از ۹۰ درصد بود. پارازیتوئیدهای ماده با عمر طولانی‌تر، نتاج نر بیشتری تولید کردند. درصد نتاج ماده تحت تأثیر مدت زمان حضور نرها قرار نگرفت. بنابراین، تغذیه نسبت به مدت زمان حضور نرها بر کیفیت پارازیتوئید تأثیر بیشتری دارد.

**واژگان کلیدی**: *Trichogramma brassicae*، تغذیه، حضور نرها، طول عمر، باروری، نرخ خروج

**Introduction**

The efficacy of biological control depends on the quality of natural enemies. To study the quality control in the parasitoids mass-reared, some laboratory experiments are conducted. Parameters that are important in the quality control include body size, longevity, fecundity, sex ratio, flight, and searching efficiency (Bai *et al.*, 1992; Pavlik, 1993; Suh *et al.*, 2000, Hoffmann *et al.*, 2001). Three groups of factors influence the quality of parasitoids (Hassan, 1994): firstly, preference of certain age, size and/or densities of host eggs (Hoffmann *et al.*, 1995; Arbab Tafti *et al.*, 2002), secondly, searching efficiency that is affected by flight ability and walking speed (Hegazi *et al*., 2000) and finally the environmental tolerance of parasitoid to temperature or humidity (Consoli & Parra, 1995). Additionally, longevity, fecundity and sex ratio of parasitoids that are important factors in quality control, are influenced by nutrition (Pavlik, 1993). The effects of several nutrients such as honey, honeydew, nectar, sucrose, fructose, glucose and protein sources have been investigated on the biological characteristics of adult parasitoids (Leatemia *et al.*, 1995; Wackers, 2001). In fact, adult feeding might have no or negative effect on these parameters (Wackers, 2001; Mansfield & Mills, 2002).

Adding to the nutrition importance, there are some studies that indicate the effect of male presence on the quality of parasitoids (Hoffmann *et al.*, 1995). It is argued that the longevity, fecundity and sex ratio of parasitoids were influenced by the number of males, mating and duration of male presence (Leatemia *et al.*, 1995; Guo, 1997; Karimian, 1998).

The *Trichogramma* species, used in inundative biological control, are well-known parasitoids. They are suitable for mass-rearing on factitious hosts because they can adapt to broad range of hosts (Smith, 1996). The performance of *Trichogramma* colonies kept in the laboratory for several generations can change over time through changes in laboratory conditions or genetic changes in colonies (Hoffmann *et al.*, 2001). Therefore, quality control is necessary for reared colonies when they are released (Smith, 1996).

The effect of adult nutrition on longevity has been reported for several *Trichogramma* species such as *T. brassicae* Bezdenko, *T. embryophagum* (Hartig), *T. minutum* Riley, *T. pretiosum* Riley and *T. japonicum* Ashmead (Leatemia *et al.*, 1995; Ram *et al.*, 1997; Attaran *et al.*, 2000; Karimian & Sahragard, 2000; Mills & Kuhlmann, 2000; Akbarzadeh Shoukat, 2006). Karimian & Sahragard (2000) demonstrated that the maximum longevity of *T. brassicae* fed on honey was 8 days. In addition, honey-fed females parasitized more host eggs. For instance, feeding honey to the females *T. nubilale* Ertle & Davis increased their parasitism 2-folds over unfed females (Olson & Andow, 1998). Attaran *et al.* (2000) and

Karimian & Sahragard (2000) obtained similar results for *T. brassicae* in Iran. Furthermore, many researchers reported that sex ratio of progeny was influenced by adult nutrition. When females were fed with honey, they produced less female progeny than unfed females even though their longevity increased. Long-lived females produced broods with more male-biased sex ratio (Hoffmann *et al.*, 1995; Leatemia *et al.*, 1995; Attaran *et al.*, 2004).

On the other hand, there are different results about the effects of male presence on *Trichogramma* quality. Karimian (1998) demonstrated that mating had no effect on the oviposition period of *T. brassicae.* Moreover, unmated *T. minutum* females produced male progeny (Leatemia *et al.*, 1995). Number of mating influenced sex ratio of *T. japonicum* and subsequent increase in male progeny (Guo, 1997)

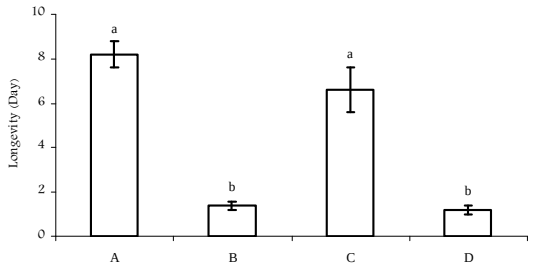
In this study, we investigated the effects of honey feeding and/or availability of males as two factors on the quality of *T. brassicae* since it is the most dominant species in Iran (Karimian, 1998; Pentureau *et al.*, 1998). It is expected that improvement of quality of parasitoids reduces the number of individuals required for every release.

**Materials and methods**

Initial population of *T. brassicae* used in the study was provided by Plant Protection Office, Shahrekord, Iran. To have access to host fresh eggs throughout the experiments, a colony of *Sitotroga cerealella* Olivier was established in the laboratory at 22 ± 2°C, 50 ± 5% RH and 10: 14 h L: D. Prior to main experiments, parasitoids were cultured for 3-4 generations at 25 ± 2°C, 60 ± 5% RH and 16: 8 h L: D. Each treatment consisted of 20 pairs (one male plus one female) of newly emerged parasitoids confined in 1.5 × 10 cm test tubes. Totally, four treatments were selected as: females presented with male for 24 h, either with or without honey; and females presented with males throughout their life span, again either with or without honey. In the treatments that feeding was required, a streak of 20% honey solution was provided as food on the inner side of the tubes. To determine the longevity, mortality of parasitoid was recorded daily for each treatment. Then parasitoids were presented with 150 of the above-mentioned fresh host eggs daily till female parasitoids died. All experiments were conducted at 25 ± 2°C, 60 ± 5% RH and 16: 8 h L: D. The parasitized eggs were also kept at the same condition, each one in separate tubes. The blackened host eggs were counted and recorded as fecundity of related parasitoid for each treatment. Afterward, the emergence rate of progeny and sex ratio were calculated. The data was subjected two-way ANOVA and means were separated by Duncan's Multiple Range Test.

**Results**

The longevity was not significantly influenced by interaction between adult nutrition and male presence (F = 0.06; df = 1; P = 0.81). Adult females of *T. brassicae*, provided with honey and either male presence for 24 h or continuously, lived longer than the unfed females (fig. 1). This indicates that female nutrition was more important than male presence. Furthermore, female longevity decreased when males were continuously presented but it was not significant compared to the 24 h male presence (nutrition: F = 99.23; df = 1; P = 0.0001 and male: F = 2.16; df = 1; P = 0.161).



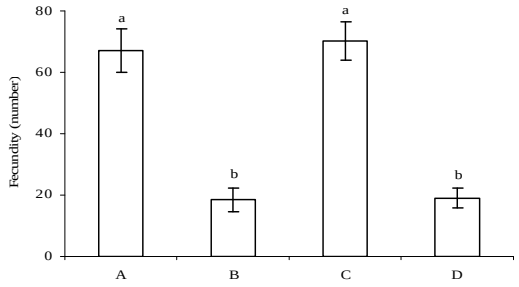

A: honey + 24 h male B: no honey + 24 h male
C: honey + continues male D: no honey + continues male

**Figure 1.** Effect (mean ± SE) of feeding and male presence on female longevity of *T. brassicae*. Means followed by different letters are significantly different (P < 0.01, n = 20).

Fecundity was not influenced by interaction between nutrition and male presence (P > 0.01). Fecundity of the honey-fed adults increased approximately 4-folds compared to the unfed females. Also, the longer females lived the more eggs produced (fig. 2). Therefore, fecundity was more influenced by feeding (F = 88.14; df = 1; P = 0.0001). There were no significant differences (F = 0.13; df = 1; P = 0.725) in fecundity when male was presented for 24 h or continuously (18.4 ± 4 eggs compared to 19 ± 3.2 eggs in the unfed females or 67 ± 7 eggs compared to 70.2 ± 6.3 eggs in the honey-fed females).

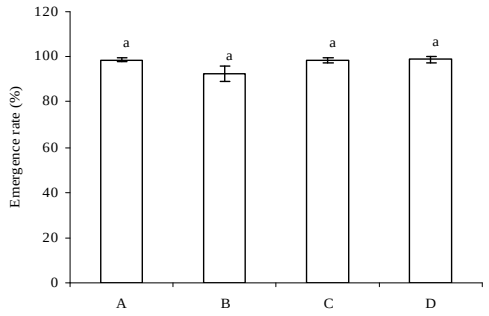
Honey and male presence did not significantly affect emergence rate (nutrition: F = 1.92; df = 1; P = 0.185 and male: F = 2.03; df = 1; P = 0.174). Emergence rate was about 90% for the entire treatments (fig. 3). The results showed no interaction between nutrition and male presence on emergence rate (F = 2.36; df = 1; P = 0.144).

Results indicated that nutrition and male presence did not have significant interaction on sex ratio (P = 0.76). Moreover, the honey-fed females produced a significantly lower proportion of female progeny than the unfed ones whether males were presented for 24 h or continuously (F = 21.47; df = 1; P = 0.0001). Additionally, male presence did not affect percentage of female progeny for the unfed and honey-fed females (F = 0.16; df = 1; P = 0.697) (fig. 4).



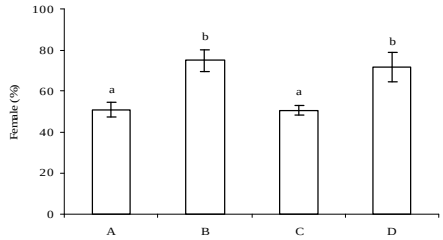

A: honey +24 h male B: no honey + 24 h male
C: honey + continues male D: no honey + continues male

**Figure 2.** Effect (mean ± SE) of feeding and male presence on female fecundity of *T. brassicae*. Means followed by different letters are significantly different (P < 0.01, n = 20).




A: honey + 24 h male B: no honey + 24 h male
C: honey +continues male D: no honey + continues male

**Figure 3.** Effect (mean ± SE) of feeding and male presence on emergence rate of *T. brassicae*. Means followed by different letters are significantly different (P < 0.01, n = 20).




A: honey + 24 h male B: no honey + 24 h male
C: honey + continues male D: no honey + continues male

**Figure 4.** Effect (mean ± SE) of feeding and male presence on female progeny of *T. brassicae*. Means followed by different letters are significantly different ($P < 0.01$, $n = 20$).

**Discussion**

The longevity of *T. brassicae* increased about 5-folds by feeding on honey, and this finding is in consistence with other researches related to this species (Attaran *et al.*, 2000; Karimian & Sahragard, 2000), or the other ones i.e. *T. pretiosum* (Mills & Kuhlmann, 2000), *T. cacoeciae* Marchal (Hegazi *et al.*, 2000) and *T. carverae* Otman & Pinto (Gurr & Nicol, 2000). The adult females of *T. brassicae* provided with honey lived 8.06 days in comparison with 2.04 days for the unfed females (Attaran *et al.*, 2000). Furthermore, the longevity of various honey-fed populations of *T. brassicae* supplied from north of Iran was between 4.34-7.6 days (Attaran *et al.*, 2004). Mansfield & Mills (2002) reported that honey-fed *T. platneri* Nagarkatti lived 2.6 ± 0.1 days whereas unfed *T. platneri* lived 2.0 ± 0.1 days meaning the quality of the parasitoidism was improved and therefore it may be possible to a reduction in the number of parasitoids. This is required for a release as flight and searching ability of parasitoids increase further thus they can find more host eggs (Leatemia *et al.*, 1995).

Continuous presence of male did not affect the longevity of the females in our study. However, Hoffman *et al*. (1995) demonstrated that with continuous access to male, longevity of *Trichogramma ostriniae* (Peng & Chen) females decreased from 13.9 to 12.1 days. Females prefer to mate with virgin males. When the females met the unmated males, they copulated quickly. Consequently, when a male is continuously presented, it is not expected that the number of mating and parasitoid quality increase (Guo, 1997). The shorter longevity may be hypothesized by some propositions. Continuous presence of male may induce

competition for food. Also, it reduces female energy because of their resistance for re-mating that causes their reduced longevity.

Providing females with honey increased fecundity (see fig. 2). Fecundity of the unfed and honey-fed *T. brassicae* was 19.6 and 56.7 eggs, respectively (Attaran *et al.*, 2000). Karimian & Sahragard (2000) demonstrated that the number of eggs produced per each unfed and honey-fed *T. brassicae* adults was 51.21 and 87, respectively. Number of parasitized eggs for honey-fed *T. embryophagum* was 41.5, whereas without nutrition it was 17.8 (Akbarzadeh Shoukat, 2006). Similar results were recorded for other close species (Leatemia *et al.*, 1995; Hegazi *et al.*, 2000). On the other hand, Lundgren *et al.* (2002) reported that 8% sucrose solution did not increase fecundity of *T. brassicae* because the solution was pure and dried quickly, hence the parasitoids could not feed from it. However, several results show that adult feeding improves biological control programs because the number of eggs produced per female is positively related to female longevity. Consequently, potential fecundity of females cannot be realized when they are released in field without nutrition, since their longevity decreases (Hassan & Zhang, 2001). Male presence did not affect fecundity, i.e. the number of eggs produced per honey-fed female, when male was present either for 24 h or continuously, was 67 ± 7 and 70.2 ± 6.3, respectively (fig. 2). Similar result was recorded for *T. ostriniae*. Fecundity of *T. ostriniae* females was 86 eggs when males were present for 24 h. Whereas it was 97.9 eggs with continuous presence of males (Hoffmann *et al.*, 1995).

Females of *Trichogramma* species with longer longevity are known to produce more males probably due to sperm depletion (Leatemia *et al.*, 1995; Kuhlmann & Mills, 1999). Providing females of *T. brassicae* with honey decreased proportion of female progeny to 66.67% compared to 83.82% for the unfed females (Karimian & Sahragard, 2000). In addition, 52.57-72.76% of progeny was female for various populations of *T. brassicae* adults fed on honey (Attaran *et al.*, 2004). In spite of lower proportion of female progeny in honey-fed parasitoids, this does not mean that adult nutrition decreases parasitoid quality. In fact, honey-fed females produced more eggs than the unfed ones (approximately 67 ± 7 and 70.2 ± 6.3 eggs compared to 18.4 ± 4 and 19 ± 3.2 eggs). Moreover, 50% of progeny were female for honey-fed group whereas it was 75% for the unfed group. In total, the number of female progeny per honey-fed females was more than the unfed ones (approximately 35 females compared to 15 females per female). Therefore, it should be considered that adult nutrition is not omitted because of lower proportion of female progeny. More fecundity in honey-fed parasitoid can compensate this limitation.

In present study, sex ratio was not influenced by period of male presence. When males were presented either for 24 h or continuously, the female progeny of honey-fed female was 51.06 ± 3.4% and 50.64 ± 2.3%, respectively, in comparison with 75.16 ± 5.3% and 71.72 ± 7.1% per unfed female. Guo (1997) reported that the number of males or mating did not affect parasitism, except sex ratio that was influenced by those factors as the proportion of female progeny decreased. This difference could be due to the number of males used in this experiment compared to the above study. More males influenced number of mating because of virgin male whereas continuous presence of male did affect number of mating.

Finally, results from this study indicated that adult nutrition improves the quality of parasitoid and male presence is not as important as nutrition.

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